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Marginal nutritional status of zinc, iron, and calcium increases cadmium retention in the duodenum and other organs of rats fed rice-based diets

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Abstract

Dietary minerals Zn, Fe, and Ca are antagonistic to Cd absorption. We showed earlier that rats fed a rice-based diet with a marginal content of these nutrients absorbed more Cd than rats fed adequate Zn–Fe–Ca (Environ. Sci. Technol., 36 (2002) 2684–2692). The present experiment was designed to determine the effects of marginal dietary Zn, Fe, and Ca on the uptake and turnover of Cd in the gastrointestinal tract. Two groups of weanling female rats (six per treatment) were fed a diet containing 40% cooked, dried rice containing 0.6 mg Cd/kg. The diet of one group contained adequate Zn (35 mg/kg), Fe (30 mg/kg), and Ca (5000 mg/kg), while that of the other group contained marginal Zn (6 mg/kg), Fe (9 mg/kg), and Ca (2500 mg/kg). Rats were fed for 5 weeks and then orally dosed with 1 g of diet containing rice extrinsically labeled with ^{109}Cd. From 0.25 to 64 days after dosing, ^{109}Cd and total Cd concentrations were determined in intestinal segments. Shortly after dosing, ^{109}Cd, as a percentage of the dose, was about 4 times higher in the duodenum of marginally fed rats than in that of control rats (10% vs 40%, respectively). Sixty-four days after dosing, ^{109}Cd was 10 times higher in marginally fed rats than in controls; however, of the amount at day 1, $<0.1\%$ remained at day 64. After 5 weeks, the concentration of elemental Cd in the duodenum of the marginally fed rats was 8 times higher than that of control rats (24μg/g dry wt. vs 2.9 μg/g dry wt., respectively). Cd concentrations in liver and kidney were 5 times higher in the marginally fed rats than those in controls (liver, 0.81 μg/g dry wt. vs 0.14 μg/g dry wt.; kidney, 4.7 μg/g dry wt. vs 0.92 μg/g dry wt., respectively). These data suggest that marginal intakes of Zn, Fe, and Ca cause the accumulation of Cd in the duodenum, which results in a greater rate of Cd absorption and a greater accumulation in the internal organs. Results are discussed in relation to mineral nutrient status and risk assessment of Cd in natural food sources.
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1. Introduction

Cadmium (Cd) is generally considered a toxic element. Food is the major source of Cd with an estimated average intake of 70 to 140 μg/week for adults in the United States (Gartrell et al., 1986; Gunderson, 1995; Pennington et al., 1986). In the past, the concentration of Cd in food has been considered the overriding risk factor that determines body burden of this element. However, as studies have shown, there are numerous other factors that affect the intestinal absorption and organ retention of Cd. Perhaps the most important one is the interaction between Cd and other mineral nutrients that affect its absorption. It has been known for some time that feeding high concentrations of zinc (Zn), iron (Fe), and/or calcium (Ca) to animals reduces the rate of absorption of Cd from various food sources (Brzóska and Moniuszko-Jakoniuk, 1998; Evans et al., 1970; Ferguson et al., 1990; Fox et al., 1979; Fox, 1983, 1988; Kello and Kostial, 1977; Koo et al., 1978). It has also been shown that humans with low-Fe status (Åkesson et al., 2002; Flanagan et al., 1978; Shaikh and Smith, 1980) and rats with low-Ca intake (Kello et al., 1979) will have an enhanced absorption of Cd. Recently it was shown that the rates

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of absorption and whole-body retention of dietary Cd increased 7- to 10-fold when experimental animals were fed diets containing marginal concentrations of Zn, Fe, and/or Ca (Reeves and Chaney, 2001, 2002). Although the concentrations of these essential minerals in the diet could be important factors that influence the extent of absorption and tissue accumulation of food-Cd, this has received little attention when decisions have been made about the potential risk of food-Cd to humans.

In previous studies, we found that a marginal nutrient status of Zn, Fe, and/or Ca in rats increased Cd absorption from foods, which seemed to be related to the transit time of Cd through the gut. We defined the term "marginal" as that concentration of Zn, Fe, or Ca in the diet that would be less than the requirement for the rat set by the National Research Council (1995) but not low enough to initiate frank deficiencies, such as severe weight loss. The fecal excretion of ^{109}Cd from an oral dose of diet containing Cd was delayed in rats that were marginally deficient in Fe and Ca, compared with rats adequate in Fe and Ca (Reeves and Chaney, 2001, 2002). When Zn also was marginal in the diet, the delay of Cd excretion was even more pronounced. Importantly, these diets contained Cd concentrations that could be found in natural foods (0.25–0.45 mg/kg), not toxicological concentrations as used in most studies of this nature. To determine how marginal mineral status might affect gut retention time of dietary Cd, we performed the present study. The general hypothesis was that diets marginal in Zn, Fe, and Ca would cause Cd to accumulate in the gut enterocytes and that Cd transit down the intestinal tract would be delayed, compared with that in rats fed adequate minerals. Cd retention by different segments of the small intestine and by the liver and kidneys was determined. We used ^{109}Cd to track Cd redistribution over time. The dietary Cd concentration in this experiment was kept near that normally found in some foods that contain a natural abundance of Cd.

2. Materials and methods

This study was approved by the Animal Use Committee of the USDA-ARS, Grand Forks Human Nutrition Research Center and was in accordance with the guidelines of the National Institutes of Health on the experimental use of laboratory animals (National Research Council, 1996).

The experiment assessed two groups of 60 female rats each (strain SAS:VAF (SD); Charles River/Sasco, Wilmington, MA) beginning at 3 weeks of age. One group was fed a diet containing all nutrients in amounts that equaled or exceeded the recommendations for the laboratory rat (National Research Council, 1995). Another group was fed a similar diet, but this diet contained only 60%, 27%, and 50% of the recommended amounts for Zn, Fe, and Ca, respectively. Descriptions of the diet formulations are given below.

The rats were fed their respective diets for 40 days; then six rats from each group were anesthetized with a 1.37:1 mixture of ketamine:xylazine (1 μL/g body wt., IP) (Ketamine HCl (Ketaset); Fort Dodge Animal Health, Fort Dodge, IA; xylazine (Xyla-Ject); Phoenix Scientific, St. Joseph, MO), and blood (~10 mL) was withdrawn from the abdominal aorta until the rat expired. The small intestine was removed and divided into five parts: one 10-cm segment that included the duodenum, three 20-cm segments of the jejunum, and one 20-cm segment that included the remaining jejunum plus the ileum (Kohn and Barthold, 1984). The lumen of each segment was rinsed thoroughly with 20 mL of ice-cold saline (0.85% NaCl in deionized water). Each segment was blotted of excess liquid and weighed to the nearest 0.01 g. Total liver and both kidneys were removed and weighed to the nearest 0.1 g. All tissues were stored at −20°C until analyzed for Zn, Fe, Ca, and Cd contents.

The remaining rats were fasted between 2100 and 0500 h; then each rat was fed 1 g of its respective diet that had been extrinsically labeled with 3 μCi (11.1×10^4 Bq) of ^{109}Cd (see Diet labeling, with ^{109}Cd). When the rats had finished consuming the labeled diet (about 1 h) they were returned to their normal dietary regimen. At 0.25, 0.5, 1, 2, 4, 8, 16, 32, and 64 days after receiving the ^{109}Cd-labeled diet, six rats from each group were killed, as described above, and tissues were taken for the determination of ^{109}Cd, Zn, Fe, Ca, and Cd. The rinsed and blotted intestinal segments were assayed immediately for ^{109}Cd by using a scintillation counter (Cobra II; Packard Instrument Co., Downers Grove, IL). The livers and both kidneys of each animal were homogenized separately in four parts saline to one part tissue; 2 mL of the homogenate were removed for the determination of ^{109}Cd contents. All tissues were stored at −20°C until analyzed for Zn, Fe, Ca, and Cd contents.

2.1. Rice processing

Polished rice (Kokuho Rose, unenriched, US No. 1 extra fancy, medium grain; Nomura & Co., Inc., Burlingame, CA) was purchased from a local food store. By analysis, the rice contained 7.3 μg Cd/kg, 10 mg Zn/kg, 2 mg Fe/kg, and 50 mg Ca/kg of edible grain. Before the rice was incorporated into the diet, it was cooked according to package directions, frozen at −80°C, and lyophilized until the moisture content was less than 10%. The dried rice was ground to a fine powder before it was incorporated into the diet. To obtain a reasonable but higher than normal amount of Cd in the finished product, we incorporated enough

$CdCl_2$ into the cooking water to obtain ~0.60 mg Cd/kg of cooked, dried, ground rice (measured concentration was 0.62 mg/kg).

2.2. Diet

The compositions of the diets are shown in Table 1. The formulations of the diets were similar to that of the AIN-93G-EGG diet described by Reeves (1996) except that 50% of the carbohydrate source (starch and sucrose) and 5% of the protein source (egg white solids) were replaced with the cooked, dried rice. The source of fat was soybean oil at 7% of the diet. The mineral supplements to the basal diet were balanced so that none of the minerals in question would be far less than or far in excess of the dietary requirements for the laboratory rat (National Research Council, 1995). Zn, Fe, and Ca in the marginal diet were supplied partially by the rice; however, even at 40% of the diet, the rice did not supply enough of any of the three minerals to obtain the desired "marginal" amount. Thus, extra minerals were added as zinc carbonate, ferrous sulfate, and calcium carbonate to bring them to the marginal amounts. In the adequate diet, the concentrations of Zn, Fe, and Ca were adjusted by adding more of the mineral salts to obtain the concentrations found in the AIN-93G diet (Reeves et al., 1993). Chemical analysis showed that the adequate diet contained 33.2 ± 0.7 mg Zn/kg, 31.6 ± 1.7 mg Fe/kg, and 4780 ± 150 mg Ca/kg (mean ± SD). The marginal diet contained 7.3 ± 0.4 mg Zn/kg, 9.6 ± 1.7 mg Fe/kg, and 2480 ± 51 mg Ca/kg. These values represented approximately 60%, 27%, and 50% of the requirements of Zn, Fe, and Ca, respectively, for the growing rat as recommended by the National Research Council (1995). The Zn concentration in the adequate diets was deliberately set higher than the requirement because this is the amount used in the standard experimental diet for laboratory rodents, AIN-93G (Reeves et al., 1993). Chemical analysis showed that the diets contained 0.26 ± 0.01 mg Cd/kg; the unfortified rice supplied only 1.4% of this amount.

Table 1
Diet composition

Dietary variable Zn, Fe, Ca	Diet 1 Adequate[a]	Diet 2 Marginal
Ingredients (g)		
Corn starch	53.25	61.5
Cooked rice[b]	400.0	400.0
Egg white, dried	150.0	150.0
Hydrolyzed starch	132.0	132.0
Sucrose	100.0	100.0
Soybean oil	65.0	65.0
α-Cellulose	42.0	42.0
Mineral mix[c]	35.0	35.0
Vitamin mix[d]	10.0	10.0
Choline bitartrate	2.5	2.5
Biotin premix[e]	1.0	1.0
TBHQ mix[f]	1.0	1.0
Zn premix[g]	1.0	0.0
Fe premix[h]	1.0	0.0
Calcium carbonate	6.25	0.0
Total (g)	1000	1000

[a] Adequate and marginal amounts of test elements as analyzed (±Zn, 33/7.3 mg/kg diet; ±Fe, 32/9.6 mg/kg diet; ±Ca, 4780/2480 mg/kg diet). The diet contained 0.26 mg Cd/kg.

[b] Kokuho Rose, US No. 1 extra fancy, medium grain rice. The rice was cooked, freeze-dried, and ground to a powder before it was incorporated into the diet. Cadmium was cooked into the rice to obtain 0.60 mg/kg dry weight (actual analysis, 0.62 ± 0.01 mg/kg).

[c] The basal mix contained the following minerals in g/kg mix: $CaHPO_4$, 227.72; KH_2PO_4, 86.2; K_2SO_4, 46.61; MgO, 13.36; $Na_2SiO_3 \cdot 9H_2O$, 1.45; $FeSO_4 \cdot 7H_2O$, 1.293; $ZnCO_3$, 0.153; $MnCO_3$, 0.318; $CuCO_3 \cdot Cu(OH)_2$, 0.257; $CrKSO_4 \cdot 12H_2O$, 0.275; H_3BO_3, 0.082; NaF, 0.064; $NiCO_3$, 0.032; NH_4VO_3, 0.007; $(NH_4)_2MoO_4$, 0.008; LiCl, 0.018; KIO_3, 0.01; Na_2SeO_4, 0.011; powdered sucrose, 622.132.

[d] AIN-93-VX (Reeves et al., 1993).

[e] Biotin premix contained 1.8 g d-biotin plus 998.2 g corn starch.

[f] TBHQ mix contained 50 g *tert*-butylhydroquinone (an antioxidant) plus 950 g soybean oil.

[g] Zn premix contained 44.1 g $ZnCO_3$ plus 955.9 g corn starch.

[h] Fe premix contained 124.4 g $FeSO_4 \cdot 7H_2O$ plus 875.6 g corn starch.

2.3. Diet labeling with ^{109}Cd

A 65-g aliquot of each diet was suspended in an equal amount of water. To each diet suspension was added 195 μCi (721.5×10^4 Bq) of ^{109}Cd. The suspension was mixed thoroughly and allowed to stand at 4°C for 12 h. Then the suspensions were frozen at −80°C and lyophilized. The dried material was thoroughly mixed and aliquots were analyzed for ^{109}Cd to assure equal distribution of the label in the dried diet. A 1-g sample was then fed to each rat by dispensing it into a glass food container that had been acid-washed.

2.4. Sample analysis

The procedures used to prepare and analyze samples for mineral content were similar to those outlined by Reeves and Chaney (2001). To assure adequate quality control, samples of bovine liver with certified concentrations of minerals were analyzed with each batch of tissues (Cd, 500 ± 30 ng/g; Zn, 127 ± 16 μg/g; Fe, 184 ± 15 μg/g; Ca, 116 ± 4 μg/g (National Institutes of Standards and Technology, Gaithersburg, MD)). Representative assayed values were within the acceptable ranges: Cd, 515 ± 14 ng/g; Zn, 124 ± 13 μg/g; Fe, 190 ± 21 μg/g; and Ca, 122 ± 6 μg/g (mean ± SD, $n = 6$).

2.5. Statistical analysis

The data were analyzed by using StatView (Version 5.0) or SAS (Version 8.02) computer programs (SAS

Institute, Inc., Cary, NC). Differences between treatment means were determined by using the Student's t statistic and were assumed to be significant if $P<0.05$. A one-component exponential decay model was used to estimate the rate of Cd turnover in the duodenum. The nonlinear regression procedure in SAS/STAT was used to estimate the coefficients for this model. A multiple regression technique was used to test whether the estimated values of the coefficients in the model were significantly different ($P<0.05$) between the two diets. Because the nonlinear model was used, the standard errors of the regression coefficients are asymptotic approximations.

3. Results

Although the rats consumed a marginal amount of dietary Zn, Fe, and Ca for a period of 40 days, the mineral concentrations were not low enough to produce an overt deficiency of Zn and Ca. The concentrations of Zn in serum, duodenum, and liver were not significantly affected by marginal intakes of dietary Zn; however, Zn was significantly ($P<0.05$) higher in the kidneys of rats fed the marginal diet (Table 2). The concentrations of Ca in serum, liver, or kidney were not significantly affected by marginal intakes of dietary Ca (Table 2). However, duodenal Ca was significantly ($P<0.01$) lower in rats fed the marginal amount of Ca than in rats fed adequate Ca.

Table 2
Marginal deficiencies of Zn, Fe, and Ca affect the mineral content of serum, duodenum, liver, and kidney

Tissue	Dietary minerals	
	Adequate Zn, Fe, Ca	Marginal Zn, Fe, Ca
Serum (μg/mL)		
Zn	1.17±0.06	1.25±0.04
Fe	3.02±0.18	1.43±0.35*
Ca	105±1	102±3
Duodenum (μg/g dry wt.)		
Cd	2.92±0.37	23.9±2.3*
Zn	103±4	104±3
Fe	151.8±7.9	57.1±0.9*
Ca	458±13	305±17*
Liver (μg/g dry wt.)		
Cd	0.14±0.01	0.81±0.06*
Zn	69.0±3.4	76.2±2.9
Fe	496±30	194±13*
Ca	104±4	112±7
Kidney (μg/g dry wt.)		
Cd	0.92±0.07	4.68±1.51*
Zn	86.7±3.2	99.8±1.1*
Fe	261±22	172±7*
Ca	236±5	229±9

Values are mean±SE for six replicates per mean. Rats were fed their respective diets for 40 days; then six rats from each group were killed and the organs removed for mineral analyses. Dietary treatment means with an * are significantly different ($P\leqslant0.05$) as determined by Student's t test.

On the other hand, feeding marginal amounts of Fe lowered tissue concentrations of Fe significantly. Serum Fe in marginally fed rats was only half ($P<0.01$) that of the adequately fed rats (Table 2). However, an analysis of hemoglobin content of blood at day 40 showed no significant effect of marginal Fe intake, suggesting that the animals were not anemic (13.0±0.72 g/dL vs 12.9±1.2 g/dL). Liver Fe of rats fed marginal Fe was only about 40% ($P<0.01$) of that of rats fed adequate Fe (Table 2). Similarly, kidney Fe was only 65% ($P<0.01$) and duodenal Fe only 30% ($P<0.01$) of those fed adequate Fe. Body weight gain was not affected by marginal intakes of any of the three minerals (data not shown).

After the blood, gastrointestinal tract, liver, and kidneys had been removed, each remaining carcass was assayed for ^{109}Cd content by using the whole-body counting technique (Reeves et al., 1994). Then the amounts of ^{109}Cd found in the whole liver and both kidneys were summed and added to the amount in the carcass (Fig. 1). ^{109}Cd in rats fed diets containing marginal amounts of Zn, Fe, and Ca peaked at 12 h where a little more than 4.0% of the dose was retained. After 64 days, they still retained about 1.7% of the dose. ^{109}Cd in rats fed adequate minerals peaked at 24 h, but these animals also accumulated about 4% of the dose. However, by 64 days, only 0.2% of the dose remained.

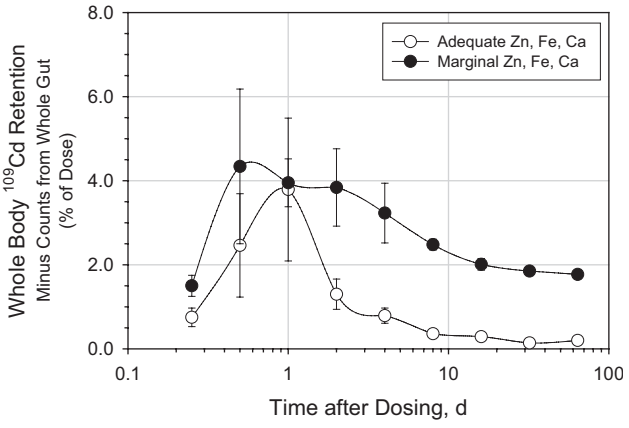

Fig. 1. Mineral nutrient status affects the retention of an oral dose of ^{109}Cd in the whole body minus the intestinal tract. Rats were fed diets with adequate or marginal amounts of Zn, Fe, and Ca for 40 days. They were fasted overnight and then fed 1.0 g of their respective diets that contained 3 μCi of ^{109}Cd. Beginning 6 h later, six rats from each group were killed at various intervals and the intestinal tracts were removed. The amount of ^{109}Cd remaining in each carcass, including the liver and kidneys, was determined by whole-body counting techniques and expressed as a percentage of the original dose. Each datum point is the mean±SE for six replicates.

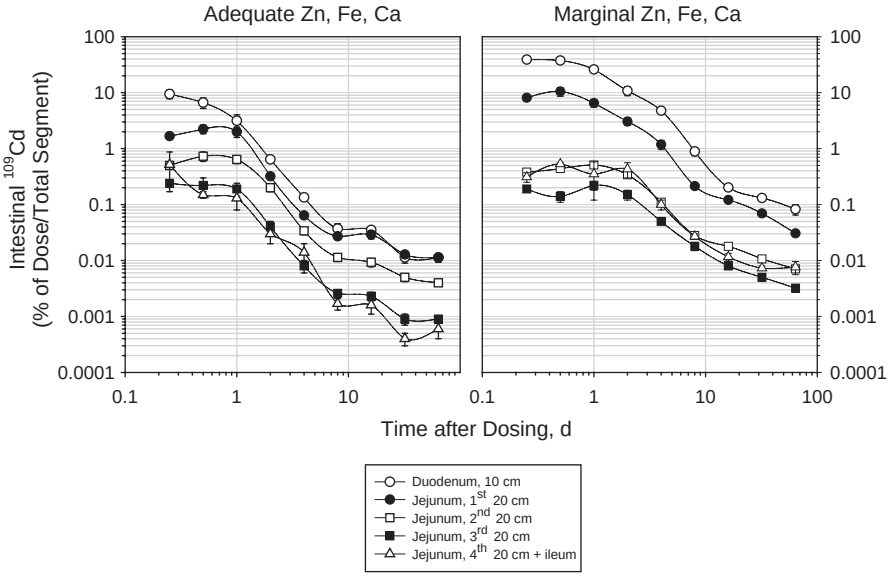

Fig. 2. Effects of mineral nutrient status on the ^{109}Cd in intestinal segments of rats fed diets labeled with the isotope. Rats were treated as outlined in the legend of Fig. 1. Beginning 6 h after the ^{109}Cd dose, six rats from each group were killed at various intervals; the small intestines were removed and the contents rinsed out with cold saline. The intestine was divided into five segments and the amount of ^{109}Cd retained in each segment over time was determined and expressed as a percentage of the original dose. Each datum point is the mean ± SE for six replicates.

Most of the ^{109}Cd was retained by the duodenum in the first few days after dosing (Fig. 2). Twelve hours after dosing, duodenal segments from rats fed marginal Zn, Fe, and Ca retained nearly 40% of the dose, whereas duodenal segments from rats fed adequate minerals retained only about 8%. After 12 h, there was a precipitous drop in duodenal ^{109}Cd to less than 1% of the dose after only 8 days in rats fed marginal Zn, Fe, and Ca and less than 0.05% in control rats. A one-component exponential decay model, $\%\text{Dose} = b_1 * e^{-\ln(2)^* t/t^{1/2}}$, was used to fit the data from 0.25 to 64 days in each duodenal curve (Fig. 3). The turnover rate of ^{109}Cd of rats fed the marginal diet was much slower than that in rats fed the adequate diet (half-life, 1.07 ± 0.12 days vs 0.47 ± 0.09 days, respectively; $P < 0.05$). Twelve hours after dosing, the first 20 cm of the jejunum of marginally fed rats retained about 10% of the dose, while adequately fed rats retained only about 2% of the dose. Here too, the turnover of the label seemed to be less in the marginally fed rats than in the adequately fed rats; however, a curve fit analysis similar to that used for the duodenum showed that the turnover rate in the proximal jejunum was similar between groups (data not shown). Each of the remaining segments retained miniscule amounts of the dietary ^{109}Cd (Fig. 2).

When we analyzed the amount of ^{109}Cd remaining in the liver and kidneys over time, the patterns of accumulation were somewhat different from that in the intestine (Fig. 4). In rats fed marginal Zn, Fe, and Ca, total liver ^{109}Cd gradually increased to a peak of about 1% of the dose at day 8 and then gradually declined over the next 56 days to about 0.6% of the dose. Total kidney ^{109}Cd continued to increase throughout the study. There was a similar pattern in rats fed adequate minerals, but the amount at day 8 was only 0.15% of the dose in liver and 0.09% in kidney.

The concentrations of Cd in the intestine were affected by the mineral content of the diet (Fig. 5). Duodenal Cd concentrations in rats fed the marginal diets were about seven times higher than those in control rats (23 μg/g dry wt. vs 3 μg/g dry wt., respectively). From day 4 to day 64, the concentration of Cd in the duodenum of the marginally fed rats decreased from 23 μg/g to about 7 μg/g; however, Cd in other segments did not decrease proportionally. Overall, the amount of Cd in other intestinal segments was higher in marginally fed rats than in those fed adequate minerals. The amount of Cd in the jejunal segments was only about 1–10% of that in the duodenum.

The concentration of Fe in the intestine showed an unusual pattern (Fig. 6) and might have been caused by the fasting/refeeding regimen. Before the oral dose of ^{109}Cd was given, the concentration of Fe in the duodenum of nonfasted rats fed an adequate supply of Zn, Fe, and Ca was about 150 μg/g dry wt. Then the rats were fasted overnight, given the oral dose of dietary ^{109}Cd, and allowed to resume normal feeding. Six hours later, the amount of Fe in the duodena of fasted/refed rats had dropped to 70 μg/g dry weight. Then at 12 h, it had rebounded to 200 μg/g and remained there until the end of the experiment. Duodenal Fe in nonfasted, marginally fed rats was less than half ($P < 0.01$) that of rats fed an adequate amount of minerals and did not

show the drop in concentration on fasting/refeeding. Overall, there was as much as three times more Fe in the duodenum of rats fed adequate Fe than those fed marginal Fe. Other segments of the intestine, such as the distal jejunum and ileum, were less affected by marginal Fe deficiency (Fig. 6).

The concentrations of Cd in livers of rats fed the marginal diet were six times higher than those in livers of rats fed adequate mineral diets, and these concentrations did not change much over time (Fig. 7). Kidney Cd in the marginal rats was about four times higher than that in the controls; however, between days 32 and 64, there was a large increase in kidney Cd in the marginal rats, with a lesser increase in the control rats (Fig. 7). During the final days of the experiment, both liver Fe and kidney Fe in both treatment groups increased two- to three-fold over those of the earlier periods (Fig. 8). This was especially true for rats fed the marginal amounts of Zn, Fe, and Ca.

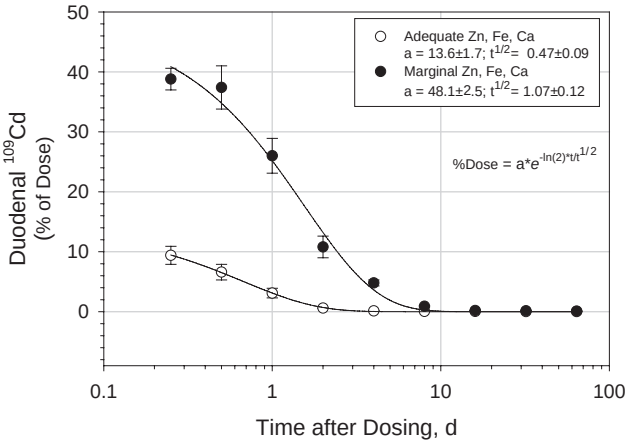

Fig. 3. Determination of the half-life of ^{109}Cd in the duodenum of rats fed diets labeled with the isotope. Rats were treated as outlined in the legend of Fig. 1. Beginning 6 h after the ^{109}Cd dose, six rats from each group were killed at various intervals; the small intestines were removed and the contents rinsed out with cold saline. The amount of ^{109}Cd retained in the duodenal portion was determined and expressed as a percentage of the original dose. Each datum point is the mean ± SE for six replicates. To determine the half-life of the labeled dose, data for each treatment group were fitted to the equation shown on the graph. Values calculated for the half-life of each group are shown in the key.

4. Discussion

4.1. Experimental results

This experiment was conducted to extend a previous observation that the fecal excretion of ^{109}Cd from an oral dose of diet containing Cd was delayed in rats that were marginally deficient in Zn, Fe, and Ca compared with rats that were not deficient (Reeves and Chaney, 2002). Fox et al. (1984) also had noted that Cd seemed to be taken up by the small intestine and remain there for a number of days, and the ^{109}Cd in this pool could be either depleted by absorption or lost from the gut by sloughing of the enterocytes. An intestinal Cd pool with a long half-life has become evident over years of research. McLellan et al. (1978) demonstrated this in a study to determine Cd absorption from an oatmeal breakfast fed to human volunteers. They added both poorly absorbed radioactive chromium (^{51}Cr) and ^{115m}Cd to the test meal to measure the absorbed ^{115m}Cd after all the ^{51}Cr had been excreted. They found that ^{51}Cr was not a good marker for Cd absorption because it was excreted very quickly, whereas ^{115m}Cd could still be detected in transit in the lumen of the gut

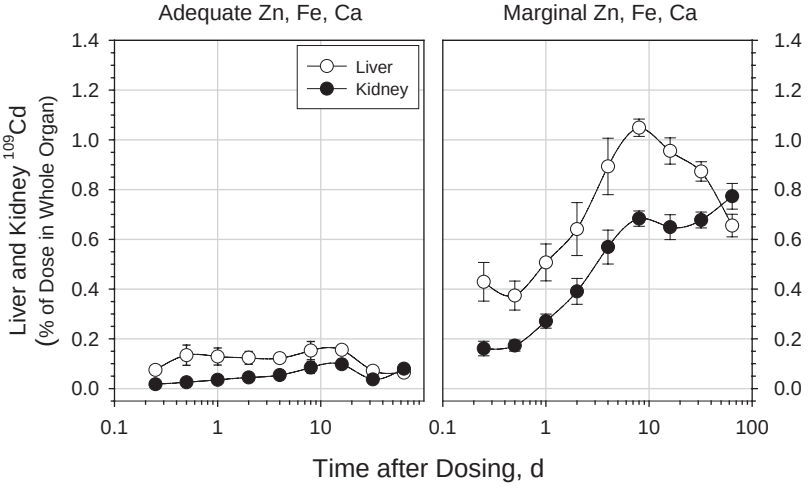

Fig. 4. Effects of mineral nutrient status on the ^{109}Cd content of whole liver and both kidneys of rats fed diets labeled with the isotope. Rats were treated as outlined in Fig. 1. Beginning 6 h after the ^{109}Cd dose, six rats from each group were killed at various intervals and the whole liver and both kidneys were removed. Each organ was homogenized in cold saline (one part tissue to four parts saline), and 2 mL was assayed for ^{109}Cd. Total organ values were calculated and expressed as a percentage of the original dose. Each datum point is the mean ± SE for six replicates.

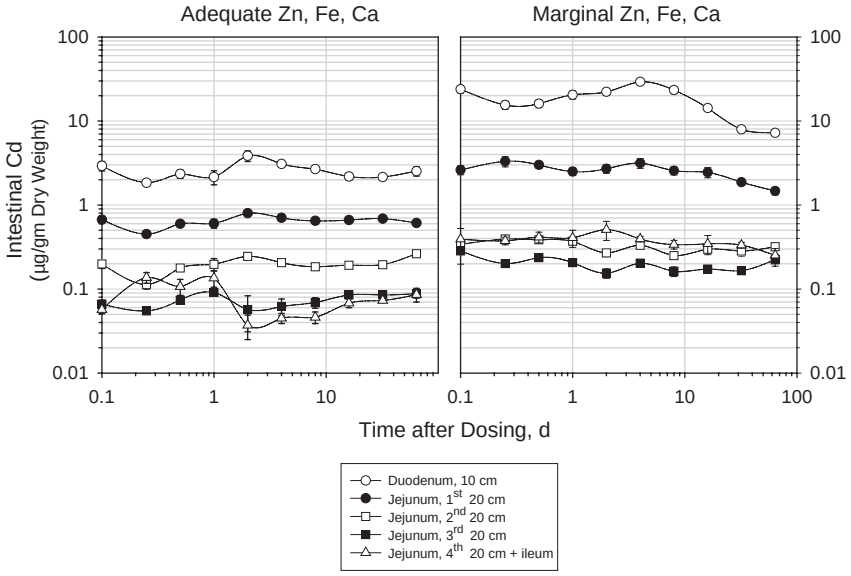

Fig. 5. Effects of mineral nutrient status on the ^{109}Cd content of intestinal segments of rats. Rats were treated as outlined in Fig. 1. Beginning 6 h after the ^{109}Cd dose, six rats from each group were killed at various intervals; the small intestines were removed and the contents rinsed out with cold saline. Each intestine was divided into five segments and the amount of Cd retained in each segment was determined and expressed as µg Cd/g dry wt. Each datum point is the mean ± SE for six replicates.

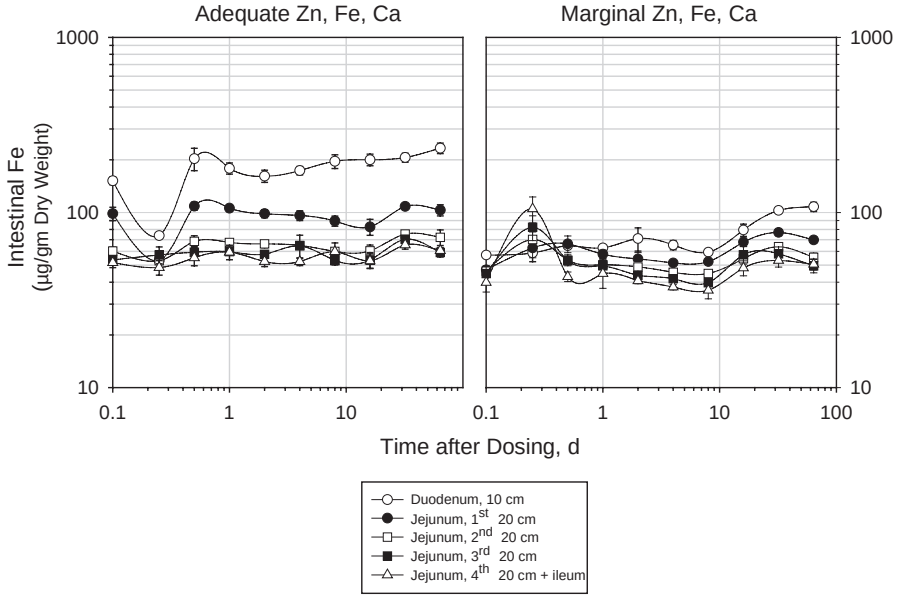

Fig. 6. Effects of mineral nutrient status on the Fe content of intestinal segments of rats. Rats were treated as outlined in Fig. 1. Beginning 6 h after the ^{109}Cd dose, six rats from each group were killed at various intervals; the small intestines were removed and the contents rinsed out with cold saline. Each intestine was divided into five segments and the amount of Fe retained in each segment was determined and expressed as µg Fe/g dry wt. Each datum point is the mean ± SE for six replicates.

up to 5 weeks after the test meal was given. Later Shaikh and Smith (1980) reported that, as they followed whole-body ^{109}Cd for a much longer time than was possible with ^{115m}Cd, three pools were evident; these were believed to be the digestate pool, the long-half-life kidney Cd pool, and a mid-half-life pool in the intestine.

Based on these studies, we believed that a study of marginal vs adequate Zn, Fe, and Ca nutriture, which measured Cd kinetics in the small intestine, might provide clearer evidence of the possibility that the main effect of marginal Zn, Fe, and Ca is to increase Cd uptake into enterocytes and induce a longer turnover time for Cd in these cells. This, in turn, would lead to higher net Cd absorption. Based on the current results, it seems that the delay in Cd retention time was caused by the sequestration of large amounts of the label in the

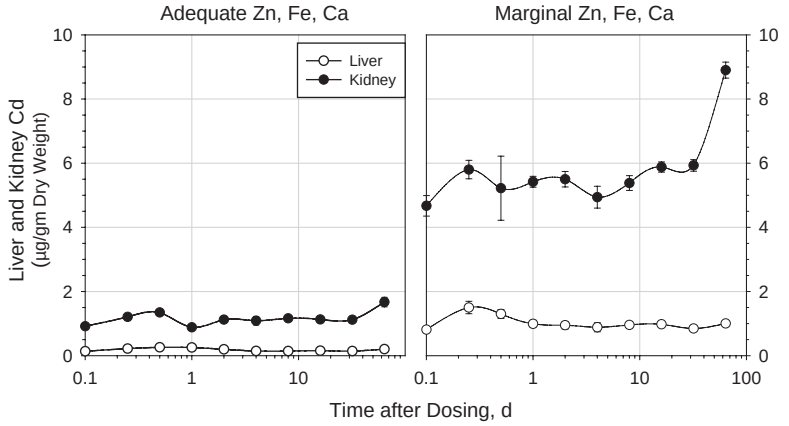

Fig. 7. Effects of mineral nutrient status on the Cd content of liver and kidneys of rats. Rats were treated the same as outlined in the legend of Fig. 1. The livers and kidneys were excised and homogenized in four parts saline to one part tissue; 2.5 mL of homogenate was analyzed for the amount of Cd retained. Organ Cd concentrations were calculated and expressed as µg Cd/g dry wt. Each datum point is the mean ± SE for six replicates.

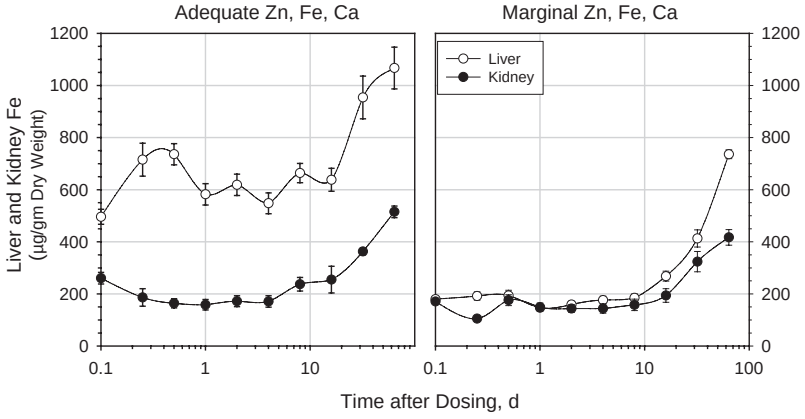

Fig. 8. Effects of mineral nutrient status on the Fe content of liver and kidneys of rats. Rats were treated the same as outlined in the legend of Fig. 1. The livers and kidneys were excised and homogenized in four parts saline to one part tissue; 2.5 mL of homogenate was analyzed for the amount of Fe retained. Organ Fe concentrations were calculated and expressed as µg Fe/g dry wt. Each datum point is the mean ± SE for six replicates.

mucosal cells of the duodenum, which retarded its transit down the intestinal tract. A half-life determination in the current study showed that the turnover rate of ^{109}Cd in rats fed marginal amounts of Zn, Fe, and Ca was slower than that of rats fed adequate amounts of these minerals. The duodenal cells tenaciously retain Cd and lose it only as these cells slough off and pass out of the body. It seems, too, that if ^{109}Cd were recycled it was not substantially retained in the distal segments of the intestine.

In our studies, we have followed the advice of Fox et al. (1979) who stressed that the absorption of Cd should be studied at concentrations relevant to food exposures. In the past, most investigations on Cd absorption have used acutely or chronically toxic doses that far exceeded those found in foods. We support the arguments of Fox et al. (1979) because if Zn and Ca competitively inhibit Cd uptake into enterocytes, then very high concentrations of Cd in the diet result in higher ratios of Cd:Zn and Cd:Ca than would occur in "normal" diets. An interesting test of Cd distribution in the intestine of rats was reported by Elsenhans et al. (1994b) who fed rats diets with 100 mg Cd/kg for several weeks and then measured Cd along the intestinal tract. In that design, it seemed as though the intestinal binding capacity had been saturated; Cd had accumulated throughout the small intestine with somewhat higher concentrations near the distal end. In comparison, results of the present study with diets with 0.26 mg Cd/kg showed that the duodenum accumulated most of the Cd and that the distal portion of the gut had very little.

Why such an excess of Cd accumulated in the duodenum of rats marginally deficient in Zn, Fe, and Ca is not understood. The current theory about how Cd is taken up by the enterocytes is that it uses the same transporter protein as Fe, DMT1 (Bannon et al., 2003; Park et al., 2002). It has been shown repeatedly that when dietary Fe is low, the DMT1 transporter is up-regulated (Fleming et al., 1999; Park et al., 2002). Because Cd can use this transporter and because the

ratio of Cd to Fe is increased in marginal diets, Cd could compete for binding sites on the transporter more effectively, and more Cd could be taken up by the duodenal enterocytes.

Although the duodenal enterocytes of marginal rats accumulated large amounts of Cd, most of the Cd was not transported to the circulation. Shortly after the ^{109}Cd dose was given to the marginal rats, nearly 40% of it was found in the duodenum; however, at the same time, less than 0.5% of the dose had passed to the liver and kidneys. This suggests either that Cd is sequestered in the enterocytes or that the transport mechanism to pass Cd out of the cell into the blood is very inefficient. The most likely candidate for Cd sequestration is metallothionein (MT). Cd is a potent inducer of MT synthesis; however, because the dietary Cd concentration was quite low (0.26 mg/kg), it seems unlikely that the initial acquisition of Cd by the enterocytes was by MT induction. The rat at weanling age will consume no more than 2 g of diet per meal, about 11 g/day. This would amount to only 0.52 μg Cd per meal, or 2.8 μg/day, or about 0.8 μmol/L in the digestive fluid when dilutions with drinking water and gastric and pancreatic secretions are considered. However, after the cells begin to accumulate the metal, MT might then be induced. Unfortunately, we did not measure MT in this experiment. Nonetheless, if the accumulation of Cd was caused by an increase in MT, one might expect that mucosal Zn would increase also because Zn readily binds to MT. However, Zn did not accumulate in the duodenum in the current study. Also, Elsenhans et al. (1994a) did not find an increase in mucosal Zn in rats consuming a much larger amount of Cd than did our rats and with a 10-fold induction of MT.

There were some rather unusual temporal effects on the tissue concentrations of Fe. When rats were fasted before the Cd dose was administered, duodenal Fe dropped to 60% of normal 6 h after they were refed but then returned to normal. Liver Fe concentration was increased by about 30% in a similar time frame and returned to normal a short time after refeeding. In rats fed adequate minerals, duodenal Fe was twice as high as that in the lower part of the jejunum. After the rats had reached the age of 12 weeks, both liver and kidney Fe concentration began to increase and remained high until the experiment was terminated at 16 weeks. We have no explanation for these occurrences at this time.

The present findings suggest designs for other studies to further characterize the regulation of the turnover pool of Cd in animals with marginal Zn–Fe–Ca and the full range of this response. Our animals were given marginal diets that were carefully designed to maintain normal growth rates. If we had provided diets that caused severe anemia (as occurs with rice subsistence diets), would more Cd have been taken up by the enterocytes and would the turnover have been further prolonged? Similarly, although it is believed that Cd enters the enterocytes via the DMT1 transporter (Bannon et al., 2003; Park et al., 2002), there is reason to believe that some Cd could use the Zn transporters also. Transporters for both Fe and Zn uptake are upregulated during Fe and/or Zn deficiency (Costello et al., 1999). However, in our study where a factorial design was used, marginal dietary Zn increased Cd uptake by enterocytes only when Fe and/or Ca were also marginal (Reeves and Chaney, 2001). This opens the possibility that Cd and Zn are competing for an Fe transporter.

4.2. Implications for risk assessment of Cd toxicity during mineral malnutrition

Questions about risks from Cd in foods and in contaminated soils remain. We have been investigating the role of subsistence rice diets on risk from Cd-contaminated soils. In general, the only human populations harmed by dietary Cd have been rice farmers who grew their rice in flooded fields that were contaminated with Cd and Zn from industrial sources. Geogenic Zn sources contain about 200 times more Zn than Cd; we reported that rice grown on contaminated flooded soils has no increase in grain Zn but a large increase in grain Cd (Chaney et al., 2001a; Simmons et al., 2003). We know of no other crops that fail to accumulate Zn in edible tissues when grown on geogenic Zn–Cd-contaminated soils (Chaney et al., 1987).

Worldwide, scientists have stressed the poor nutritional quality of polished rice with respect to Zn and Fe (Graham et al., 1999; Gregorio et al., 2000; Welch and Graham, 2002). In human populations subsisting on rice, anemia is prevalent and reduces work capacity (Ross, 2002); in these populations, Zn is marginal to deficient, which can reduce the immune response (Fortes et al., 1998; Shankar and Prasad, 1998). The World Health Organization and other authorities have estimated that a large percentage of the deaths in children under the age of 5 years are related to malnutrition, including deficiencies of such micronutrients as vitamin A, Zn, and Fe (Black, 2003). This problem is so severe that an international program has been established at the Consultative Group on International Agricultural Research Centers to breed rice and other staple foods with higher density of bioavailable Fe and Zn (Graham et al., 2001; Gregorio et al., 2000; Gregorio, 2002). The unique association of rice-based diets with food-chain Cd disease in long-term residents of Cd-contaminated areas may be related to Zn and Fe malnutrition commonly found in these populations.

In contrast with rice-based diets, shellfish-containing diets with high concentrations of Cd have not been as clearly associated with human Cd toxicity. Although shellfish concentrate Cd in the edible tissues, no adverse effects have been seen in individuals who consumed

large numbers of Cd-rich oysters (Sharma et al., 1983; Sharma, 1985). Vahter et al. (1996) found no increase in blood Cd in young women who regularly consumed shellfish, even though they ingested nearly 3 times as much Cd as young women who did not regularly consume shellfish. They also found that the shellfish consumers had higher serum ferritin concentrations than the control group (Vahter et al., 1996). Oysters are rich in Zn and Fe; perhaps the increased intake of bioavailable Zn and Fe reduced excessive Cd absorption. In addition, when garden foods (Shipham, UK (Strehlow and Barltrop, 1988); Palmerton, PA, USA (Sarasua et al., 1995); Germany (Ewers et al., 1993)) or other crops grown on Cd-contaminated soils were consumed over long periods, no renal tubular dysfunction was observed, even though soils were commonly 10 times higher in Cd than the rice soils in Japan and China where a high prevalence of kidney tubular dysfunction was observed.

The present work helps explain why rice has been so important in human Cd disease. Because no other dietary exposure to a Cd source has regularly caused Cd disease, it seems that there must be a sensitizing factor in these latter populations or that the bioavailability of Cd from rice is quite different from that of other foods. Combining our results with the now recognized Zn–Fe malnutrition of subsistence rice consumers suggests that these people up-regulated their absorptive mechanisms for Zn and Fe and absorbed more Cd. Thus, because rice excludes Zn, takes up Cd, and is inherently low in Fe, it could have a unique ability to increase Cd uptake and to slow its rate of Cd turnover in the small intestine. The slower turnover rate would allow a much longer time frame for Cd release into the circulation rather than it being rapidly excreted as seen in animals with adequate Fe and Zn nutriture.

The results of our studies also raise the possibility of a simple method to prevent further Cd disease. Indeed, it is possible to remediate the contaminated soils so that rice will not contain excessive Cd, but removal and replacement of contaminated soils is very expensive: on the order of $1–2 million (US) per hectare for the 30-cm removal depth commonly needed. Most countries cannot afford to remediate large areas of rice soils by soil removal. Chaney et al. (2001b) have illustrated the use of phytoextraction of soil Cd by using rare plants that hyperaccumulate soil Cd into their shoots. Although phytoextraction is more cost effective than soil remediation, it takes much longer to complete. Recognition that malnutrition induced by subsistence rice consumption is causing much greater Cd absorption than occurs with other staple diets suggests that nutritional supplementation could reverse the risk of most individuals at risk of being harmed by soil contamination with Cd. A Zn–Fe–Ca mineral supplement, or more likely a balanced vitamin–mineral supplement appropriate for their diets, would maintain the small intestine in a state that would discourage efficient Cd absorption and that would allow the enterocyte Cd to be excreted soon after ingestion.

These findings also have implications for the design of food safety rules for Cd. If the dietary source plays such a significant role in the risk of Cd from rice, it seems evident that different foods might need different Cd limits. There is no technical basis, for example, to extrapolate the limit of 0.2 mg/kg for Cd in rice to wheat or other foods. Furthermore, although most soil Cd contamination is geogenic with 200 times more Zn than Cd, some soil contamination results from industrial use of Cd only. Cd is used without Zn in pigments, plastic stabilizers, and electroplating. Without concurrent Zn contamination, the potential for soil Cd transfer to foods is much greater, and Cd bioavailability to animals including humans might also be greater.

Finally, we stress the importance of food-level exposures of Cd and other potentially toxic elements in the study of risk assessment. Most foods except for rice contain low concentrations of Cd, which is poorly absorbed. It is not relevant or practical to use toxic doses to study food-Cd risks. The metabolic processes that seem to allow rice to induce the absorption of Cd in humans have not been observed in most other Cd research. A more comprehensive understanding of the biochemistry involved in the bioavailability of Cd from several foods would help resolve remaining questions and provide the support for a needed food safety policy with regard to food-Cd.

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